*Desalination and Water Treatment*
www.deswater.com

doi: 10.5004/dwt.2023.29623

296 (2023) 32–40
June

# Heavy metals occurrence in the pig farm environment and the effects on an anaerobic digestion process of pig manure

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Received 8 December 2022; Accepted 15 May 2023

**ABSTRACT**

The objective of this study was to assess the levels of heavy metals in animal feed, manure, and sediment in the farm environment's field water supply and drainage system. Samples of animal feed were collected from pigs at various growth stages, as well as feces and sediment samples from the discharge system leading to the Day River. An analysis of the samples found concentrations of Cu and Zn that ranged from 61 to 2,731 and 151–4,269 mg/kg·TS, respectively, which was significantly higher than the acceptable Vietnamese standards for agricultural land. The ecological risk coefficient in the estuary to the open channel potential ecological index (RI) exceeded 600, indicating a high level of ecological risk in the contaminated pig farm area. Furthermore, the study assessed the impacts of Cu, Fe, Pb, and Zn on anaerobic biodegradation of pig manure, revealing that levels of these metals from 0–40 mg/L, negatively affected biogas yield, with a decrease from 4.38% to 15.30%. Moreover, the treatment efficiency of chemical oxygen demand (COD) and total solids (TS) was affected adversely with a decline from 35.57% to 43.86%. The study found that the most impactful metals on the biogas yield were Pb, Zn, and Cu in that order, respectively. Conversely, the concentration of Fe level of 12 mg·Fe/L demonstrated a significant increase in the biogas yield and higher efficiency of TS and COD treatment compared to non-metallic samples. However, an increase in concentration from 20 to 40 mg·Fe/L resulted in an adverse effect, reducing the biogas yield. Essentially, this research was conducted to aid in management control measures to regulate the input of raw materials and feed, drinking water, and sanitation procedures implemented in the pig site. This would assist in limiting the propagation of heavy metals into the immediate environment.

*Keywords:* Heavy metals; Anaerobic digestion; Pig manure; Biogas generation

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## 1. Introduction

The rapid expansion of pig farms in rural areas of Vietnam has led to increased economic growth, creating work opportunities for farmers and augmenting household income. However, pig farming may have adverse effects on the environment surrounding these farms, owing to the potential for untreated or improperly treated pig manure and wastewater to be released into ponds, channels, sewerage, and fields, resulting in serious environmental impact, such as eutrophication of lakes and rivers, and increased greenhouse effect, soil, and crop pollution [1,2]. A pressing ecological issue is the potential for metal pollution induced by pig manure applications, which may contain elevated concentrations of heavy metals such as Cu, Zn, As, and Cd [3–5]. The heavy metals in pig manure are derived from various sources, such as groundwater contamination by Mn, Fe, and animal feed [3,4]. The accumulation of heavy metal residues in manures may persist in surface soils as a result of long-term agricultural use [6–8]. Heavy metal congestion could result in adverse effects on soil fertility and product quality [9], causing heavy metal migration through leaching and runoff [10,11]. Therefore, given the potential risks of heavy metal pollution, heavy metal residues in animal manure have raised scientific concerns [12,13].

To mitigate the environmental impact of pig farming, biogas digester systems are commonly implemented to treat pig waste, thereby reducing waste discharged into the environment. The anaerobic digestion process is critical to the microbiological decomposition of pig manure and involves specific anaerobic and facultative bacteria types, which are sensitive to heavy metals due to various physicochemical factors, including electronegativity, Pearson softness index, standard oxidation–reduction potential, the solubility-product constant ($K_{sp}$) of the metal-sulfide complex, electronic density, and covalent index [14,15]. Heavy metals such as Cu, Zn, Pb, Fe, and Mn are commonly found in waste from pig farms in variable concentrations [16]. Some metals at low concentrations, including Ni, Co, Zn, and Cu, are necessary for activating numerous enzymes and coenzymes in anaerobic digestion. However, high concentrations of heavy metals may exhibit pathological or stimulating properties for biochemical reactions [17–20]. Despite extensive research on this subject, it is still unclear how particular heavy metals inhibit or promote microbial growth. Moreover, there is still ambiguity regarding determining the appropriate dosages of heavy metals that can stimulate growth but not become toxic [21].

Numerous studies have been conducted on how heavy metals impact the anaerobic digestion process. Typically, the yield of methanogenesis granular sludge during anaerobic digestion decreases when heavy metal concentrations exceed 32 ppm [22]. Other research shows that the toxic inhibition effect varies, with a pattern of Zn > Cr > Ni ≈ Cd [11], or Cu (most toxic) > Ni ~ Zn > Pb (least toxic) [23], or Hg > Cd > Cr(III) [24]. However, no publications have assessed the impact of Zn, Fe, Pb, and Cu on the anaerobic digestion process of pig manure to date. This study aims to evaluate the selective heavy metal content in pig manure, sediment around pig farms, discharged open channels from farms to rivers, and assess environmental risk. Additionally, the study will investigate the influence of these metals on biogas production during the anaerobic digestion process of pig manure.

## 2. Materials and methods

### *2.1. Site of the research and sampling points*

The typical centralized pig farms located in Lam Dien commune, Chuong My district, Hanoi capital of Vietnam were selected for this study. The pig farms established in 2014 to control the pollution from piggery near households include 22 farms in which each farm has from 500–2,000 pig heads.

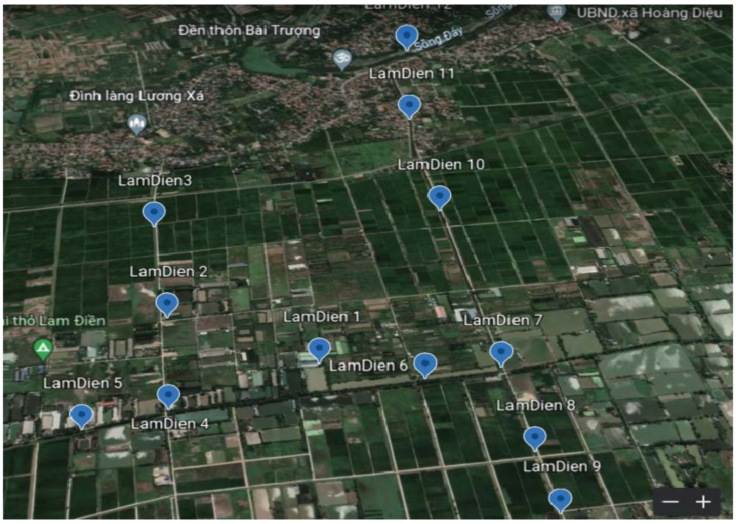
All samples were collected on Mr. Toi's farm in Luong Xa, Lam Dien, Chuong My, Hanoi (LamDien 1) to analyze the presence of heavy metals in the food and manure of the farm. There is a typical model for pig farming in Vietnam, with about 1,800 pigs/y capacity. The food samples were gathered to ensure sample representativeness following the QCVN 01–12: 2009. Three 0.5-kg food samples from different phases of pig growth according to specific nutritional requirements PF1, PF2, PF3 were collected. The pig manure samples (50 g/each) of MPF1, MPF2 and MPF3 from different pig growth phases were collected. The solid sample was from the post-biogas residue (PBR) and the sludge sample was in a small lagoon (SSL) where wastewater post-biogas was discharged before entering a canal. The sludge sample at a discharged outlet of the lagoon enters an open canal (GDC) flowing to Day River. Samples were transferred into plastic tubes, tagged, and quickly closed to prevent contamination. All samples were stored separately until laboratory analysis.

Twelve samples were collected to measure concentrations of heavy metals of sludge from the open channel from Toi's property to Dayrive. The samples are signed as LamDien 1 sample was taken at Toi's farm, the samples from LamDien 2 to LamDien 7 were taken along the primary sewage system about 100 m from discharge points of pig farms; LamDien 8 and 9 samples at a rice field and, LamDien 10, 11 and 12 samples were taken in main discharged open channel to Day River. Fig. 1 shows the map of sampling sites. The samples were collected according to TCVN 6663-15:2004 (equality to ISO 5667-15: 1999), homogenized, and subsequently stored at 4°C until the lab analysis.

In the anaerobic digestion experiments, pig manure samples (PM) were collected from Mr. Toi's farm (LamDien 1). All samples were homogenized and then kept at 4°C for experiments. Table 1 shows the main characteristics of PM. Each sample was taken in triplicate, and the averages of the three measurements are displayed.

### *2.2. Sample processing and analysis*

Dried samples were filtered through a 0.45 μm stainless steel filter, heated to constant weight, and finely powdered in an agate mortar for homogenization. Acid digestion of sludge was performed using a microwave device. 0.2–0.5 g of dry sludge was put in a Teflon vessel and microwave-digested with 10 mL of concentrated $HNO_3$ (Speedwave 4 BERGHOF). Then, the digest was cooled to room




| Sampling points | GPS position | |
|---|---|---|
| | Latitude | Longitude |
| LamDien1 | 20°52'31" | 105°42'39" |
| LamDien2 | 20°52'45" | 105°42'46" |
| LamDien3 | 20°52'47" | 105°42'57" |
| LamDien4 | 20°52'45" | 105°42'34" |
| LamDien5 | 20°52'51" | 105°42'33" |
| LamDien6 | 20°52'16" | 105°42'02" |
| LamDien7 | 20°52'16" | 105°42'10" |
| LamDien8 | 20°52'13" | 105°42'14" |
| LamDien9 | 20°52'14" | 105°42'38" |
| LamDien10 | 20°52'15" | 105°43'17" |
| LamDien11 | 20°52'15" | 105°43'33" |
| LamDien12 | 20°52'13" | 105°43'47" |

Fig. 1. Map of sampling points.

Table 1
Main characteristics of pig manure sample

| | M01 | M02 | $M_0$ |
|---|---|---|---|
| pH | 6.8 | 6.8 | 6.8 |
| Chemical oxygen demand, mg·$O_2$/L | 5,904 | 5,664 | 5,784 |
| Total solids, mg/L | 2,220 | 2,236 | 2,228 |
| Total suspended solids, mg/L | 1,062 | 1,106 | 1,615 |
| Total phosphorus, mg/L | 181 | 176 | 178.5 |
| N–$NH_4^+$, mg/L | 233 | 228 | 230.5 |

temperature and distilled water was added to achieve 50 mL of digest solution. Next, the solution was filtered by 0.45 μm filter. Finally, the heavy metal contents of the solution were determined using ICP-OES at Geological Test and Analyze Center.

### 2.3. Risk environmental analysis

Indices $I_{\text{geo}}$ was used as the accumulation level for the risk evaluation. Geoaccumulation index ($I_{\text{geo}}$) [25] is a traditional evaluation model for identifying heavy metal deposition in sediments, and it was estimated using the methodology:

$$I_{\text{geo}} = \log_2\left(\frac{C_n}{1.5B_n}\right) \tag{1}$$

where $C_n$ is the concentration of specific metal "$n$" in the samples, $B_n$ is the crustal shale background content of the metal [26]. The constant 1.5 reduces background value volatility due to lithogenic sources. There are seven classification levels of $I_{\text{geo}}$, from lower than 0 to higher than 4 [25].

This study also calculated the coefficient of metal pollution ($C_d$) and potential ecological index (RI) to evaluate the combined effect of metals on ecosystems and the environment. RI index was proposed by Hakanson (Sweden) scientist [19]; this index is calculated based on specific pollution factors ($C_f^i$), and potential ecological risk coefficient ($E_r^i$). The calculation formulas are as follows:

$$\text{RI} = \sum E_r^i \tag{2}$$

$$E_r^i = T_r^i \times C_f^i \tag{3}$$

$$C_f^i = \frac{C_o^i}{C_n^i} \tag{4}$$

$$C_d = \sum_{n=1}^{n} C_f^i \tag{5}$$

where $C_n^i$ is the reference value (mg/kg), $C_n^i$ is the calculation's needed reference value, generally the highest value of the heavy metal's pre-industrial background in sludge. In this investigation, the maximum background value of the metals in pre-industrial sediment was used: Cd = 1, As = 15, Pb = 25, Cr = 60, Cu = 30 and Zn = 80; $C_o^i$: the measured value of heavy metals in sediment (mg/kg); $T_r^i$ is the heavy metal toxicity factor as shown in Hakanson's study [19].

### 2.4. Chemicals and the anaerobic digestion system

Chemicals ($CuSO_4$, Pb $(NO_3)_2$, $ZnSO_4$, $FeCl_2$, and $K_2Cr_2O_7$) were bought from Merck Chemicals.

The anaerobic digestion system was established by conducting thirty-two 1.0 L volume anaerobic batch tests (Fig. 2). Each reactor has a volume of 1.0 L and can only be filled with biogas up to 0.7 L. The batch reactor was connected to the corresponding gas column (take note of the numbering), and the top was closed with precision. Finally, the pipe connection for gas sampling was sealed with the screw tab.

PM was fermented anaerobically under 35°C ± 2°C conditions with a total solid content of 7.35% for 25 d. The 1 L glass reactor of self-designed batch digesters with 700 g total liquid. The initial sludge samples contained volatile solids-VS (83.14%), total solids-TS (6.15%), pH (7.52), chem-

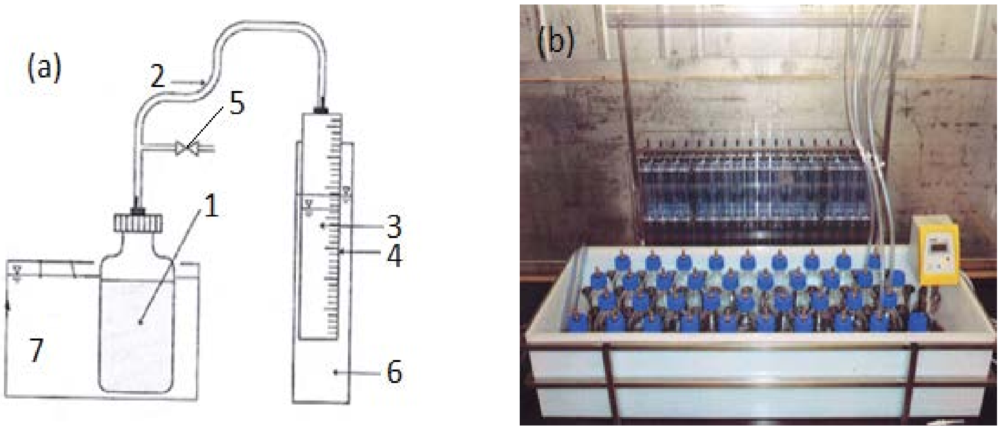



Fig. 2. (a) The anaerobic digestor: 1: glass bottle 1 L; 2: connection pipe; 3: gas column; 4: ruler; 5: valve; 6: saturated brine tank; 7: tray and (b) the anaerobic digestion system.

ical oxygen demand (COD) (6863.6 mg/L), Pb (0.688 mg/L), Fe (0.4676 mg/L), Zn (9.4296 mg/L) and Cu (1.3086 mg/L).

Salts solutions of the Cu, Zn, Pb, Fe or Mn were added to the reactors at various concentrations, as shown in Table 2. The reactors had a total compaction capacity of 0.62 L. Before the anaerobic digestion, each sample was combined for 3–5 min to achieve a homogenous mixture. Each reactor was manually agitated for 1 min daily during the anaerobic digestion procedure. The trials were done three times with the average result being recorded. Table 2 displays some label examples.

All parameters were measured according to APHA Standard Methods. A universal oven (Model UN55, Germany) was used for TS measurement following 2540. B: 2000 method, a furnace (Model B180, Nabertherm, Germany) was used for VS measurement (2540. E: 2000 method). The COD was determined at 605 nm with a spectrophotometer (Model UH-5300, Hitachi, Tokyo, Japan) according to 5220.C: 2012 method. The water displacement method was used to determine biogas volume.

## 3. Results and discussion

### *3.1. Content of heavy metal in pig feeds and manures in selected farms*

The content of Cd in all samples is minimal, while Cu, Fe, Zn (Zn highest) in pig growth phase 1 is higher than phases 2 and 3 because Cu, Fe, Zn, Pb supplement reduced against production phase 1, except Mn (Fig. 3).

Analysis of pig feed samples from farms with different herd sizes was carried out. All feed samples contained Cu, Zn and Fe, indicating that these additives have been widely applied in livestock production: Cu is often used as a growth promoter in livestock units raising pigs and poultry [27]; Zn is added to pig feed as a "panacea" while poultry needs Zn for growth, feathering, bone development and reproduction, which only requires 50 mg/kg [28]; Cd in all samples was ≤0.1 mg/kg TS, while Cu, Fe, Zn (Zn were the highest) in the samples were relatively high. For pigs growing stage 1 Cu 235 ± 13 mg/kg, Fe 235 ± 13 mg/kg, Zn 1,243 ± 68 mg/kg, Pb 40 ± 2 mg/kg and Mn 105 ± 6 mg/kg

Table 2
Sample labels

| Sample | Concentration | Sample | Concentration |
|---|---|---|---|
| $M_0$ | 0 mg/L | | |
| $Zn_{12}$ | 12 mg·Zn/L | $Pb_{12}$ | 12 mg·Pb/L |
| $Zn_{20}$ | 20 mg·Zn/L | $Pb_{20}$ | 20 mg·Pb/L |
| $Zn_{40}$ | 40 mg·Zn/L | $Pb_{40}$ | 40 mg·Pb/L |
| $Fe_{12}$ | 12 mg·Fe/L | $Cu_{12}$ | 12 mg·Cu/L |
| $Fe_{20}$ | 20 mg·Fe/L | $Cu_{20}$ | 20 mg·Cu/L |
| $Fe_{40}$ | 40 mg·Fe/L | $Cu_{40}$ | 40 mg·Cu/L |

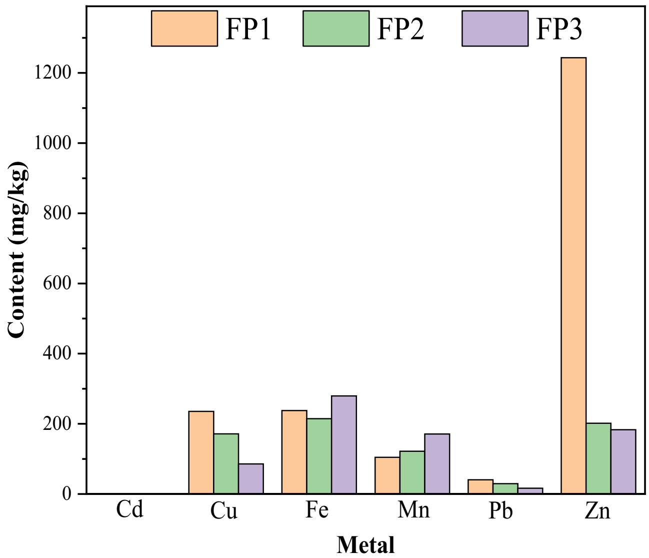



Fig. 3. Heavy metal content in pig feed.

higher than feed stage 2 (Cu 171 ± 9 mg/kg, Fe 215 ± 11 mg/kg, Zn 202 ± 11 mg/kg, Pb 29 ± 2 mg/kg and Mn 122 ± 6 mg/kg) and stage 3 (Cu 86 ± 4 mg/kg, Fe 280 ± 15 mg/kg, Zn 183 ± 10 mg/kg, Pb 17 ± 1 mg/kg and Mn 171 ± 9 mg/kg). The Cu content in pig feed varies with the herd's age, with higher Cu concentrations in weaned and pig diets than in sow feed [29,30], showing that feed tends to use more Cu additives to promote pig growth. Feed for pigs, cattle, and chickens from livestock farms in England and Wales and Jiangsu province in China contained similar levels of Cu as the feed in our study [30,31]. The amount of Zn during periods of significant variation; in England and Wales, the average Zn content of the chicken feed is 2–3 times higher than that poultry requires for healthy growth [30]. Mn content hardly changed significantly.

Fig. 4 shows that Cu, Fe and Zn in phase 1 (MPF1) pig manure are higher than MPF2 and MPF3, while Mn content is higher in MPF2.

Table 3 presents the content of six metals (Cu, Zn, Mn, Fe, Cd, Pb) in manure from the farm. Cu, Zn and Pb concentrations in growing pig manure stage 1 (MPF1) were 1,236 ± 68 mg/kg, 6,322 ± 348 mg/kg and 189 ± 10 mg/kg, respectively. The content of Mn and Fe in feces was 576 ± 31 mg/kg and 1,987 ± 109 mg/kg. At growth stage 2 (MPF2) and 3 (MPF3), metal content decreased compared to stage 1. Like feed, the highest metal content in cattle

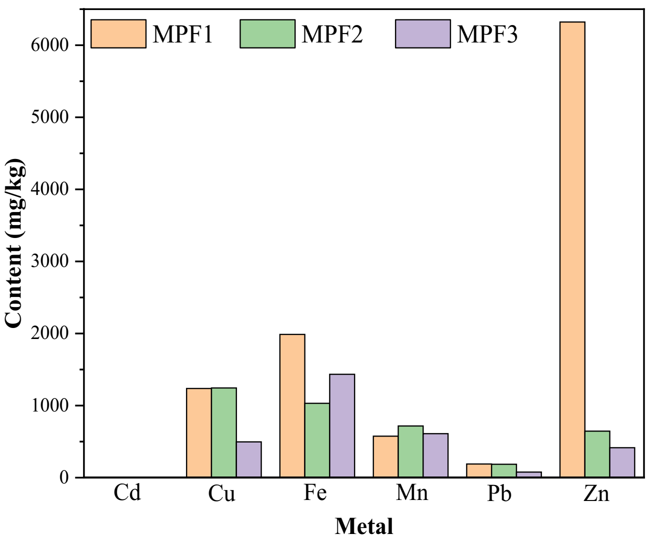



Fig. 4. Heavy metal content in pig manure.

manure was Zn and Cu. Apart from Cd, no significant differences in heavy metal concentrations were observed from the farm over the different periods. Sager [32] reported that Austria's mean Cu in pig manure was 282 mg/kg. Similarly, a fecal survey of England and Wales typically shows a typical concentration of 350 mg/kg in pig manure. However, Dong et al. [33] found a much higher mean Cu concentration of 1.018 mg/kg in pig manure from pig farms in Hangzhou, a major city in the Yangtze River Delta, China. This result shows that metal residues in feces in the study area are significantly higher than in other regions worldwide.

The concentrations of Cu and Zn in sediments (Table 4) at LamDien 1, 4, 5, 6, 9, 10, 11 sites are higher than the Vietnamese standard for agricultural soil. The distribution of Cu, Mn, Zn is higher than other sites because these points are taken along the central discharged open channel primary sewage system into Day River; At LamDien 2, 3, 7, 8 sites at the rice field, the concentration Cu, Pb, Zn are lower except for Fe.

Like antibiotics, pig farms extensively use heavy metals such as Cu, Zn, and Cd as feed additives to promote growth and prevent infections [34]. However, this use also results in the transfer of metals to manure which can contaminate the receiving environment. Therefore, in an agricultural environment lacking waste treatment facilities, assessing the amount of Cu, Zn, Mn, Fe, Cd and Pb introduced into the sediments from adjacent pig farms is necessary. This study selected a typical pig farm area to investigate the distribution pattern of Cu, Zn, Mn, Fe, Cd and Pb. The main objective of this study was to determine the concentrations of Cu, Zn, Mn, Fe, Cd and Pb in the infield drainage channel sediments affected by pig farming. The results showed that the concentrations of Cu and Zn in the sediments (Table 4) at Lam Dien locations 1, 4, 5, 6, 9, 10, 11 were higher than the Vietnamese standards for agricultural land. Cd content still shows below the maximum allowable threshold. The distribution of Cu, Mn, Zn is higher than that of other points because these points are taken along the primary sluice system of the open canal discharging into Day River, the highest Cu content exceeds 2,731/100, the Zn content exceeds 2,369/200, Pb exceeded 386/70 at location LamDien 1 (located near the farm area directly related to agricultural production); At LamDien points 2, 3,

Table 3
Heavy metal concentration of pig feeds and manures in selected farm

| TT | Samples | Metal content (mg/kg) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Cd | Pb | Mn | Fe | Cu | Zn |
| 2 | FP1 | <0.1 | 40 ± 2 | 105 ± 6 | 238 ± 13 | 235 ± 13 | 1,243 ± 68 |
| 3 | FP2 | <0.1 | 29 ± 2 | 122 ± 6 | 215 ± 11 | 171 ± 9 | 202 ± 11 |
| 4 | FP3 | 0.1 ± 0.01 | 17 ± 1 | 171 ± 9 | 280 ± 15 | 86 ± 4 | 183 ± 10 |
| 5 | MPF1 | <0.1 | 189 ± 10 | 576 ± 31 | 1,987 ± 109 | 1,236 ± 68 | 6,322 ± 348 |
| 6 | MPF2 | 0.2 ± 0.01 | 185 ± 10 | 716 ± 39 | 1,031 ± 56 | 1,245 ± 68 | 645 ± 35 |
| 7 | MPF3 | 0.2 ± 0.01 | 78 ± 4 | 610 ± 33 | 1,433 ± 78 | 496 ± 27 | 415 ± 23 |
| 8 | PBR | 0.2 ± 0.01 | 93 ± 5 | 3,596 ± 198 | 5,023 ± 276 | 627 ± 34 | 1,390 ± 76 |
| 9 | SSL | 1.0 ± 0.06 | 301 ± 16 | 1,412 ± 77 | 6,485 ± 357 | 2,084 ± 114 | 3,121 ± 172 |
| 10 | GDC | 1.1 ± 0.06 | 386 ± 21 | 1,007 ± 55 | 8,374 ± 461 | 2,731 ± 150 | 4,269 ± 235 |

Table 4
Metal contents in open canal sediment from pig farms to Day River

| Samples | Metal contents (mg/kg) | | | | | |
|---|---|---|---|---|---|---|
| | Cd | Cu | Fe | Mn | Pb | Zn |
| LamDien 1 | 1.1 ± 0.05 | 2,731 ± 143 | 8,374 ± 440 | 1,007 ± 52 | 386 ± 20 | 4,269 ± 224.43 |
| LamDien 2 | <0.1 | 54 ± 3 | 35,311 ± 1,856 | 539 ± 28 | 41 ± 2 | 165 ± 8.67 |
| LamDien 3 | 0.1 ± 0.01 | 50 ± 3 | 39,085 ± 2,054 | 624 ± 32 | 43 ± 2 | 152 ± 7.99 |
| LamDien 4 | <0.1 | 765 ± 40 | 27,565 ± 1,449 | 734 ± 38 | 168 ± 8 | 2,458 ± 129.22 |
| LamDien 5 | 0.3 ± 0.02 | 333 ± 17 | 4,372 ± 229 | 447 ± 23 | 71 ± 4 | 1,232 ± 64 |
| LamDien 6 | <0.1 | 930 ± 49 | 28,531 ± 1499 | 491 ± 25 | 194 ± 10 | 2,990 ± 157 |
| LamDien 7 | 0.1 ± 0.01 | 55 ± 3 | 36,740 ± 1931 | 517 ± 27 | 46 ± 2 | 151 ± 7 |
| LamDien 8 | <0.1 | 56 ± 3 | 36,947 ± 1,942 | 625 ± 32 | 46 ± 2 | 167 ± 9 |
| LamDien 9 | <0.1 | 693 ± 36 | 35,205 ± 1,850 | 554 ± 29 | 169 ± 8 | 2,305 ± 121 |
| LamDien 10 | 0.4 ± 0.02 | 365 ± 19 | 32,711 ± 1,719 | 473 ± 24 | 97 ± 5 | 1,346 ± 70 |
| LamDien 11 | 0.8 ± 0.04 | 322 ± 17 | 33,409 ± 1,756 | 488 ± 25 | 93 ± 5 | 1,161 ± 61 |
| LamDien 12 | <0.1 | 61 ± 3 | 26,923 ± 1,415 | 438 ± 23 | 35 ± 2 | 231 ± 12 |

7, 8 in the rice field, the Cu, Pb, Zn contents were all lower except for Fe.

### 3.2. Risk assessments of selected heavy metals in pig farming environment

The geographical accumulation index ($I_{geo}$) in Fig. 5 demonstrates that all of the samples are 'medium pollution to strong pollution' by Zn, Cu, Pb and Cd are above the profound $I_{geo}$ level of 'moderate contamination and even reach three values of $I_{geo}$ (designating "heavily contaminated"). According to calculations using $I_{geo}$'s math, a significant order of relative risk for a few heavy metals is Zn and Cu > Pb > Cd based on calculation $I_{geo}$'s math (Fig. 5).

The $I_{geo}$ index depends mainly on the heavy metal content of the soil at the site of use. Therefore, a common site for the sediment was chosen as the mud use point for the assessment. Fig. 5 shows the $I_{geo}$ values for all analyzed samples. PBR showed a small risk of high environmental contamination for all the sediment cases analyzed. SSL and GDC samples show a very high risk of environmental contamination. It is because Zn, Cu, and Pb in SSL and GDC samples are all at very high contamination levels, and PBR samples are at high levels. As for the Cd in the PBR sample, which is lower than the pollution risk threshold, the SSL and GDC samples show a moderate contamination risk. According to calculations using $I_{geo}$'s math, a significant order of relative risk for a few heavy metals is Zn & Cu > Pb > Cd based on calculation $I_{geo}$'s math (Fig. 5).

#### 3.2.1. Contamination factors ($C_f^i$) and the degree of contamination ($C_d$)

Table 5 shows the $C_f^i$ pollution factors and the $C_{\text{mud samples}}$. In general, all samples have high levels of pollution, mainly caused by elements of C Cu, Pb and Zn ($C_f^i > 6$, $C_d > 32$)

The study also investigated pollution assessment based on single and aggregate factors. The single pollutant assessment indicated similarity with the $I_{geo}$ index assessment, as

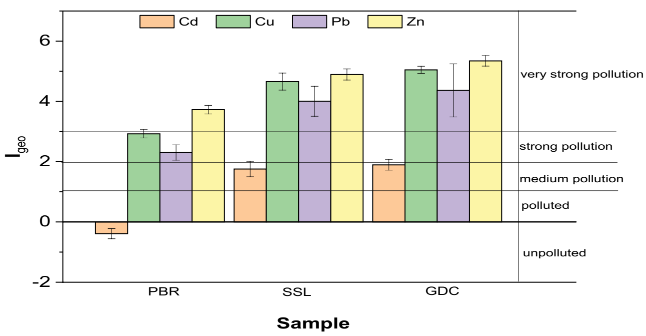



Fig. 5. Geoaccumulation index ($I_{geo}$) of selected metals in pig manure.

Table 5
Contamination factors ($C_f^i$) and the degree of contamination ($C_d$)

| Samples | $C_f^i$ | | | | $C_d$ |
|---|---|---|---|---|---|
| | Cd | Cu | Pb | Zn | |
| SSL | 1.01 | 69.45 | 12.05 | 39.02 | 121.53 |
| GDC | 1.12 | 91.03 | 15.46 | 53.36 | 160.97 |
| PBR | 0.23 | 20.90 | 3.71 | 17.37 | 42.21 |

all samples exhibited Cd levels below the pollution threshold. However, the metal concentrations of Cu, Pb, and Zn in the samples were all very high, except for Pb in the PBR sample, which was at a medium level. The GDC sample showed a risk of Cu exceeding 91.03/6 times the highest pollution risk, whereas Pb and Zn metals exceeded 15.46/6 and 53.36/6 times the highest pollution risk, respectively. The evaluation of aggregate contamination level for Cd also indicated that the samples were at a very high contamination risk, as Cd levels in the PBR sample exceeded >200%, the SSL sample exceeded >600%, and the GDC sample exceeded >800% of the highest infection contamination risk level.

*3.2.2. Ecological risk assessment*

Calculations of the potential ecological risk coefficient and the risk index of the metals in the study area are shown in Table 6. The results indicate a very high risk in the study area, especially at the lagoon outlet to the open canal with RI > 600. These may result from accumulating metals for a long time without wastewater treatment from the farm.

### *3.3. Effects of heavy metals on pig manure's anaerobic digestion process*

*3.3.1. Effects of lead, chromium, zinc, and copper on the production of biogas*

Typically, 0.2 to 1.11 m$^3$ of biogas was obtained when 1 kg of dry organic matter was decomposed [14]. However, numerous aspects of anaerobic fermentation significantly impacted how much biogas was produced. The biogas output in studies using original pig dung samples and additional metal samples is shown in Table 7. The experimental results (Table 7) demonstrated that, in comparison with the sample without supplemental metals ($M_0$), all supplemental metal samples (Zn, Cu, and Pb) resulted in a decrease in the total volume of biogas. $Zn_{12}$ sample was cut down by 6.89%, while $Zn_{20}$ sample was 11.45%. According to a report from Tereza Dokulilova, the effects of Zn concentration on the anaerobic digestion process of animal waste in the range from 12, 50 and 100 mg/L reduced the methane generation capacity 6.3% ± 2.5% after 21 d [15]. For Pb metal, biogas volume generated in all 3 supplemented samples in the range from 12, 20 and 40 mg·Pb/L is decreased when Pb concentration increased: $Pb_{12}$ (6.28%), $Pb_{20}$ (9.39%), $Pb_{40}$ (15.30%). These results are similar to other work when Pb in waste-activated sludge will affect biogas produced from the anaerobic co-digestion process [16]. In Cu-supplemented samples, it can be seen that the biogas generated efficiency has come down from 4.38%, 5.53%, 6.54% to $Cu_{12}$, $Cu_{20}$, $Cu_{40}$ samples, respectively. In the anaerobic treatment of rich organic wastewater, the presence of $Cu^{2+}$ ion$^s$ will inhibit the microorganism activities resulting in low removal efficiency of COD concentration [17]. While the samples supplement Fe, the biogas produced is more significant than samples without Fe. It has the highest volume of the Fe-supplemented sample ($Fe_{12}$). The effect of ferrous (added as $FeCl_2$) on the anaerobic co-digestion of Phragmites straw and cow, according to Zhang et al. investigations [18], enhanced the cumulative biogas outputs by 18.1% by extending the peak time with high daily biogas yields. When considering the entire fermentation process, prolonging the gas production peak stage and improving cellulase activities were primarily responsible for the effect of $Fe^{2+}$ addition on biogas yields. This event showed that the creation of biogas depended on the breakdown of organic compounds in the input materials. When it took enough time for microorganisms to adapt and dissolve systematically, the first vital gas phase represented the digestion of the dissolving organic compounds, and the second weaker phase represented the digestion of the persistent organic compounds. It is possible to conclude that biogas yields can decrease as the concentration of heavy metals in digestion rises.

*3.3.2. Effect of copper, zinc, iron, and lead on COD and TS removal during the anaerobic co-digestion process*

The ability to produce biogas during the anaerobic co-digestion process is closely related to the efficiency and capability of COD removal. Based on the effects of Cu(II), Zn(II), Fe(II), and Pb(II) into the digestion mixture, it is possible to estimate microbial activity and evaluate the total activity of anaerobic digestion using these assays. The

Table 6
Ecological risk of metal in the study area

| Samples | $E_r^i$ | | | | RI |
|---|---|---|---|---|---|
| | Cd | Cu | Pb | Zn | |
| SSL | 30.3 | 347.25 | 60.25 | 39.02 | 476.82 |
| GDC | 33.6 | 455.15 | 77.3 | 53.36 | 619.41 |
| PBR | 6.9 | 104.5 | 18.55 | 17.37 | 147.32 |

Table 7
Impact of copper, zinc, iron, and lead on the amount of biogas produced during digestion

| Sample | $V_{biogas}$ (mL) | Sample | $V_{biogas}$ (mL) |
|---|---|---|---|
| $Zn_{12}$ | 812.14 | $Cu_{12}$ | 826.82 |
| $Zn_{20}$ | 772.35 | $Cu_{20}$ | 816.91 |
| $Zn_{40}$ | 786.77 | $Cu_{40}$ | 796.06 |
| $Pb_{12}$ | 817.62 | $Fe_{12}$ | 950.95 |
| $Pb_{20}$ | 790.80 | $Fe_{20}$ | 894.73 |
| $Pb_{40}$ | 739.64 | $Fe_{40}$ | 883.88 |
| $M_0$ | 872.20 | | |

Table 8
Effect of copper, zinc, iron, and lead on chemical oxygen demand and total solids removal during the anaerobic co-digestion process

| Sample | Total phosphorus (mg/L) | $N–NH_4^+$ (mg/L) | Total solids (mg/L) | Chemical oxygen demand (mg·$O_2$/L) |
|---|---|---|---|---|
| $M_0$ | 272 | 206 | 786 | 3,740.8 |
| $Zn_{12}$ | 279 | 209 | 818 | 3,746.4 |
| $Zn_{20}$ | 286 | 212 | 845 | 4,268 |
| $Zn_{40}$ | 287 | 201 | 799 | 4,092.8 |
| $Fe_{12}$ | 293 | 217 | 679 | 3,284 |
| $Fe_{20}$ | 267 | 264 | 713 | 3,244.8 |
| $Fe_{40}$ | 284 | 190 | 736 | 3,654.4 |
| $Pb_{12}$ | 279 | 205 | 816 | 3,991.2 |
| $Pb_{20}$ | 281 | 238 | 827 | 4,252.8 |
| $Pb_{40}$ | 286 | 229 | 833 | 4,373.6 |
| $Cu_{12}$ | 268 | 203 | 809 | 3,916.8 |
| $Cu_{20}$ | 279 | 210 | 795 | 4,081.6 |
| $Cu_{40}$ | 282 | 224 | 822 | 4,204.8 |

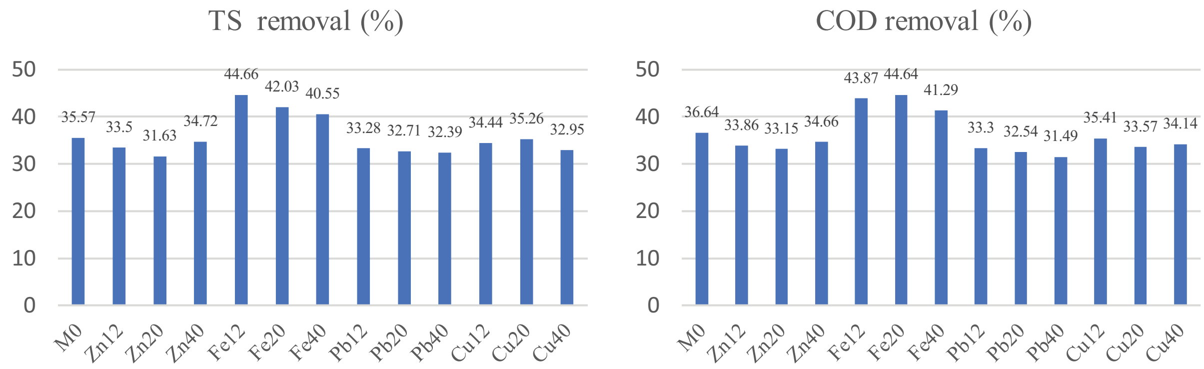



Fig. 6. Total solids and chemical oxygen demand removal efficiency.

findings of COD and TS analyses over time are presented in Table 8 and Fig. 6 with a sampling frequency of every 5 d. After 20 d of digestion, the supplementation of Zn, Cu, and Pb resulted in decreased TS and COD concentrations. The efficiency of TS and COD removal decreased to 35.57% and 36.64%, respectively.

Cu(II) and Zn(II) impair enzyme structure and function by attaching to thiol and other groups on protein molecules or replacing real metals for false enzyme groups. This action weakens the biomass and increases the likelihood of growth of microorganisms' enzymes, resulting in decreased COD during anaerobic digestion.

Contradictory, TS and COD removal efficiency **in** Fe-supplemented samples increased compared with $M_0$. TS removal efficiency of $M_0$ is 35.57% and TS removal efficiency of $Fe_{12}$, $Fe_{20}$, $Fe_{40}$ samples are 44.66%, 42.03% and 40.55%, respectively. The COD removal efficiencies of $Fe_{12}$, $Fe_{20}$ and $Fe_{40}$ are 43.86%, 42.71% and 41.29%, respectively, which are higher than the removal efficiency of $M_0$.

## 4. Conclusion

The occurrence of heavy metals in pig farm environments, including animal feedstocks and sediment in discharges, was investigated in this study. The concentration of Cu and Zn was found to be higher than the Vietnamese standard for agricultural soil in pig manure, biogas tanks, and sediments open channels from the pig farm to Day River. Risk assessments of selected heavy metals indicated a very high risk in the study area, particularly at the lagoon outlet to an open canal, with an RI greater than 600. This accumulation of metals is likely a result of prolonged exposure caused by a lack of wastewater treatment from the farm. Concentrations of Cu, Pb, and Zn at 12 mg/L negatively affect the anaerobic digestion process of pig manure, resulting in an overall decrease in biogas production (by 4.38%–15.30%). The level of influence of these metals is Pb > Zn > Cu, respectively. The addition of Fe had the effect of effectively increasing the cumulative biogas production (up to 18.1%). Therefore, it is strongly advised that the anaerobic co-digestion system for biogas production and the safety of land application be controlled, considering the presence of hazardous heavy metals such as Cu, Zn, Cr, and Pb in municipal sludge.

## Funding

This research was funded by The Asia Research Center at Vietnam National University, Hanoi (ARC-VNU), Korea Foundation for Advanced Studies under project number CA.20.08ª, and Korea Environment Industry & Technology Institute (KEITI) through Ecological Imitation-based Environmental Pollution Management Technology Program, funded by Korea Ministry of Environment (MOE) (2021002800001).

## Symbols

| | | |
|---|---|---|
| $I_{geo}$ | — | Geoaccumulation index |
| $C_n$ | — | Concentration of specific metal "*n*" in the samples |
| $B_n$ | — | Crustal shale background content of the metal |
| $C_d$ | — | Coefficient of metal pollution |
| RI | — | Potential ecological index |
| $C_f^i$ | — | Specific pollution factors |
| $E_r^i$ | — | Potential ecological risk coefficient |
| $C_n^i$ | — | Reference value, mg/kg |
| $C_o^i$ | — | Measured value of heavy metals in sediment, mg/kg |
| $T_r^i$ | — | Heavy metal toxicity factor |

## Acknowledgments

The authors wish to thank the Dang Viet Toi family in Lam Dien, Chuong My, Hanoi, Vietnam, who has provided pig manure and kindly support samples. The authors also thanks to Thu-Bich Do, Thi-Thuy Nguyen (VNU University of Science) and Ngoc-Khanh Nguyen (Center for Environment Verification, Environmental Police Agency) for their analysis.

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